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A. E. Brandt
Iowa State College

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Tests of Significance in Reversal or Switchback Trials

By A. E. Brandt

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

R. E. Buchanan, Director

STATISTICAL SECTION

AMES, IOWA

CONTENTS

SUMMARY

By extending "Student's" t-test to differences higher than the first, a method is provided for analyzing the results of reversal tests employing as many periods as are practical with the organism and test used. It is shown in Part II that identical results can be obtained by the methods of analysis of variance so that the investigator may at will use either the methods presented in Part I or those in Part II if but one attribute of the experimental units is measured. But, if the investigator has one or more other measures relevant to his experimental results and wishes to increase the precision of his tests through the use of covariance, the methods of Part II must be used.

Tests of Significance in Reversal or Switchback Trials*

By A. E. Brandt

In certain biological experiments, individuals are subjected to two tests or treatments, and the differences between the results are used for comparing the efficacy of the treatments. ''Student'' (6) presented a test of the significance of the mean of such differences, using the data of Cushny and Peebles to illustrate it. In some cases the comparisons thus obtained are not independent of time nor of the order of presentation, so two groups are treated or tested simultaneously but with the order of presentation in one group the reverse of that in the other. Clearly, if each group of test animals is to be subjected once to each of the two treatments, two test periods are necessary. In feeding trials with dairy cows three or more test periods are commonly used.

To analyze fully the data from such reversal tests or switchback trials, that is, to test for significance all the sources of variability, the arithmetic procedure known as analysis of variance is the most efficient statistical tool. In many reversal trials such as dairy cow feeding, however, the only pertinent source of variability is the treatment applied, and this can be tested easily and simply by Fisher's extension of ''Student's'' t-test. The tests of significance appropriate to reversal or switchback trials will be presented by means of arithmetic examples using data from experiments involving two, three and four test periods respectively. The extensions of ''Student's'' t-test for the two, three and four period experiments will be given in Part I. In Part II the results will be verified and amplified by the analysis of variance and, in the example involving four test periods, by the analysis of co-variance.

Those wishing to use the methods presented here will find it convenient to read and use only that portion which applies directly to the type of experiment at hand. If but a single characteristic has been measured, the investigator may use the method of Part I or that of Part II according to his personal

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pleasure or preference. If, however, two or more characteristics have been measured with the purpose of analysing them simultaneously, the methods of Part II must be used. What will, no doubt, appear as needless and cumbersome details of computation to the reader interested in statistical theory may appeal to the research worker as welcome directions for his guidance.

PART I.

THE EXTENSION OF "STUDENT'S" t-TEST TO REVERSAL OR SWITCHBACK TRIALS INVOLVING TWO OR MORE TEST PERIODS

EXPERIMENTS INVOLVING TWO TEST PERIODS

In experiments of this type two groups of individuals, A and B, are each subjected to two tests or treatments, X and Y, simultaneously but in the order X, Y in group A, and Y, X in group B. Problem A 102 from the Department of Applied Statistics, University College, London University, is of this type. In the experiment from which the data for this problem were obtained, 30 rabbits were taken from the stock colony and divided at random into two equal groups, A and B. The rabbits in group A were injected with a subsidiary standard insulin, those in group B with an equal amount of international standard insulin on the same day, and the percentage blood sugar reduction for each rabbit was recorded. Seven days later the rabbits in group A were injected with the international standard insulin and those in group B with the subsidiary standard insulin and the percentage blood sugar reduction for each animal again noted. The data, together with some results of calculations which aid in the analysis, are given in table 1.

TABLE 1. RABBIT INSULIN TESTS.
(Percentage blood sugar reductions.)

Rabbit number	Period I	Period II	Differences
	Group A		
	X_1	Y_2	$X_1 - Y_2$
1	29	27	2
2	39	21	18
3	45	53	— 8
4	35	22	13
5	41	35	6
6	22	21	1
7	21	27	— 6
8	29	22	7
9	38	23	15
10	40	24	16
11	51	46	5
12	41	27	14
13	35	23	12
14	32	25	7
15	32	30	2
	530	426	104

Group B

	Y_1	X_2	$Y_1 - X_2$
16	42	35	7
17	47	28	19
18	30	29	1
19	37	28	9
20	35	30	5
21	34	23	11
22	35	31	4
23	53	30	23
24	33	37	— 4
25	57	24	33
26	39	33	6
27	36	37	— 1
28	25	30	— 5
29	41	23	18
30	35	31	4
	579	449	130
Grand total	1109	875	234

Note: X = Subsidiary standard insulin
Y = International standard insulin

The mean of these differences for Group A is 6.9 and that for group B is 8.7. Because of the arrangement of the experiment the difference between these two means is in effect the difference between the mean percentage blood sugar reduction due to the two kinds of insulin. This may be stated algebraically as follows:

$$\frac{X_1 - Y_2}{15} = \text{mean group A}$$

$$\frac{Y_1 - X_2}{15} = \text{mean group B}$$

$$\frac{Y_1 - X_2}{15} - \frac{X_1 - Y_2}{15} = \text{difference of means}$$

$$= \frac{Y_1 - X_2 - X_1 + Y_2}{15}$$

$$= \frac{Y_1 + Y_2}{15} - \frac{X_1 + X_2}{15}$$

Since the means are both positive and nearly equal it seems that one would be justified, without making a statistical test, in concluding that the difference in effect of the two insulins is not significant. The test will be applied, however, as an illustration of the method.

Following the extension of "Student's" treatment of the error of the mean given by Fisher (4), from the differences are found (d = difference, n = number of individuals in the group, S means "the sum of"):

Symbol	Group A	Group B
Sd	104.	130.
$\bar{d}$	6.93	8.67
Sd^2	1582.	2690.
$(Sd)^2/n$	721.1	1126.7
$S(d - \bar{d})^2$	860.9	1563.3

$$s = \sqrt{\frac{860.9 + 1563.3}{14 + 14}} = 9.31$$

$$t = \frac{(8.67 - 6.93)}{9.31}\sqrt{\frac{(15)\ (15)}{15 + 15}}$$
$$= 0.51 \text{ (Degrees of freedom } = 28)$$

From the table of t given by Fisher (4) one finds that for 28 degrees of freedom the value 0.51 will be equaled 62 times in a hundred by chance and must therefore conclude that the difference in percentage blood sugar reduction produced by the two insulins is not significant. The test, then, verifies the conclusion drawn from inspection of the means of the differences.

EXPERIMENTS INVOLVING THREE TEST PERIODS

In an experiment involving three test periods, two groups of individuals, A and B, are each subjected to two tests or treatments, X and Y, simultaneously but in the order X, Y, X in group A and Y, X and Y in group B. To illustrate the test of significance appropriate to this type of experiment, data given by Baker (1) will be used.

In this experiment, 10 cows were selected from the Iowa State College Holstein-Friesian herd and divided into two equal groups. Care was taken to have the groups as nearly equal as possible with regard to milk production, stage of gestation, body weight, condition and age. These cows were each given 10 pounds of timothy hay and 30 pounds of corn silage daily but were fed different grain mixtures. Treatment X, then, consisted of feeding a grain mixture of 1 part of corn and cob meal to 1 part of ground oats, while treatment Y consisted of feeding a grain mixture of 4 parts corn and cob meal, 4 parts of ground oats and 3 parts of gluten feed. The three treatment periods covered 105 days—three periods of 35 days each. The yields for the first 7 days of each period were not considered because of the possible effect of the transition from one treatment to the other. The data, together with sums and differences which aid in the calculations incidental to testing, are given in table 2.

TABLE 2. GLUTEN FEED IN A TIMOTHY HAY RATION.
(Production of milk in pounds.)

Cow number	Period I	Period II	Period III	Differences
		Group A		
	X_1	Y_2	X_3	$X_1 - 2Y_2 + X_3$
493	433.0	413.7	362.9	—31.5
647	744.6	797.4	780.3	—69.9
596	858.2	753.8	680.1	30.7
560	977.2	1025.2	1007.4	—65.8
319	655.0	616.1	494.6	—82.6
Sums	3668.0	3606.2	3325.3	—219.1
		Group B		
	Y_1	X_2	Y_3	$Y_1 - 2X_2 + Y_3$
634	671.3	610.3	596.8	47.5
592	615.1	555.4	488.5	— 7.2
409	776.9	733.0	693.9	4.8
480	1101.8	958.8	939.6	123.8
485	764.4	717.6	717.0	46.2
Sums	3929.5	3575.1	3435.8	215.1
		Both Groups		
Sums	7597.5	7181.3	6761.1	

The differences given in the last column may be found by setting up a difference table for each of the 10 cows. To illustrate, differences for two cows are given in table 3. The second

TABLE 3. PORTION OF DIFFERENCE TABLE.

Cow number	Period number	Production in pounds	First differences	Second differences
	I	433.0		
			19.3	
A 493	II	413.7		—31.5
			50.8	
	III	362.9		
	I	744.6		
			—52.8	
A 647	II	797.4		—69.9
			17.1	
	III	780.3		

difference for cow A493 in table 3 is identical with her difference in table 2, and the sum of the first differences in table 3 is equal to the yield in the first period minus that in the third period. The second difference is equal to the yield in the first period plus that in the third period minus twice the yield in the second period.

A comparison based on second differences is the same in effect as a comparison of the yield in the second period with the mean of those in the first and last periods.

The result of subtracting twice the yield of milk for each cow while on ration Y from the sum of her two yields while on ration X is negative in four out of the five cases in group A, which suggests very strongly that ration Y is superior for milk production. In group B, the result of subtracting twice the yield of milk for each cow while on ration X from the sum of her two yields while on ration Y is positive in four out of the five cases which again indicates that ration Y is superior. Since the number of individuals is small, and since the variations between differences are relatively large within each group, one is scarcely justified, without a statistical test, in concluding that ration Y is superior to ration X.

To make this test it is proposed that Fisher's extension of "Student's" t-test be applied to second differences in the same manner as it was applied to first differences in the example involving two test periods. The logic of this extension is apparent if one considers that though there are three test periods there are but two rations to be compared and that the second difference is a properly weighted comparison of the yield obtained from a cow being fed one ration with one being fed the other. From the second differences, d, in column 5 of table 2 are found:

Symbol	Group A	Group B
Sd	—219.1	215.1
$\bar{d}$	— 43.82	43.02
Sd^2	17973.15	19792.01
$(Sd)^2/n$	9600.96	9253.60
$S(d - \bar{d})^2$	8372.19	10538.41

$$s = \sqrt{\frac{8372.19 + 10538.41}{4 + 4}} = 48.62$$

$$t = \frac{43.02 - (-43.82)}{48.62} \sqrt{\frac{(5)\ (5)}{5 + 5}}$$

$$= 2.82 \quad (\text{D. F.} = 8)$$

The chances that this value of t may be fortuitous are about 26 out of 1000, so it seems safe to conclude that the inclusion of gluten feed in the grain mixture fed in a timothy hay ration to Holstein-Friesian cows increased the production of milk. The average increase was 21.7 pounds per cow for a 28-day period.

EXPERIMENTS INVOLVING FOUR TEST PERIODS

As in the experiments involving less than four test periods, two groups of individuals, A and B, are each subjected to two tests or treatments, X and Y, simultaneously, but in the order X, Y, X, Y for group A and Y, X, Y, X for group B. An excellent example of this type of experiment is presented in a bulletin by Cannon, Hansen and O'Neil (2). In the experiment described in this bulletin the effect upon the production of butterfat due to watering cows from water bowls indoors or from tanks outdoors was studied.

As usual, the cows were assigned to the groups in such a way as to make the two lots as nearly comparable as possible. During the experiment two cows went dry, but luckily they were of the same breed, and one came from each group. The cows were treated alike with regard to feed, milking and exercise but were subjected to different methods of watering. While on treatment X, the cows were watered from water bowls in their stalls; while on treatment Y, from an outside tank. Each of the four test periods consisted of 35 days with no records being considered for the first 7 days of each period to escape the transition effect. The individual yields of butterfat together with the third differences are given in table 4.

TABLE 4. USE OF WATER BOWLS IN THE DAIRY BARN.
(Production of butterfat in pounds.)

Cow number	Period I	Period II	Period III	Period IV	Differences
			Group A		
	X_1	Y_2	X_3	Y_4	$-X_1 + 3Y_2 - 3X_3 + Y_4$
906	40.66	31.59	27.83	14.47	—14.91
750	27.39	19.34	19.52	13.57	—14.36
787	34.57	26.52	30.65	25.23	—21.73
796	30.14	28.14	27.05	25.52	— 1.35
933	37.31	29.79	32.24	27.10	—17.56
Sums	170.07	135.38	137.29	105.89	—69.91
			Group B		
	Y_1	X_2	Y_3	X_4	$-Y_1 + 3X_2 - 3Y_3 + X_4$
749	50.83	54.96	39.71	39.70	34.62
817	31.62	31.91	32.37	27.84	— 5.16
675	35.83	38.02	29.08	30.71	21.70
833	31.72	30.48	24.63	25.39	11.22
763	20.62	22.37	14.75	15.40	17.64
Sums	170.62	177.74	140.54	139.04	80.02
			Both Groups		
Sums	340.69	313.12	277.83	244.93	10.11

The third differences given in column six may be found readily by setting up a difference table for each of the 10 cows. To illustrate, differences for two cows are given in table 5.

Clearly the third differences obtained in this manner are identical with those in table 4 which were obtained by substituting in the following formulas:

$$d = -X_1 + 3Y_2 - 3X_3 + Y_4, \text{ for Group A}$$

and

$$d = -Y_1 + 3X_2 - 3Y_3 + X_4, \text{ for Group B}$$

TABLE 5. PORTION OF DIFFERENCE TABLE.

Cow number	Period number	Pounds of butterfat	Differences		
			First	Second	Third
A 906	I	40.66			
			—9.07		
	II	31.59		5.31	
			—3.76		—14.91
	III	27.83		—9.60	
			—13.36		
	IV	14.47			
A 750	I	27.39			
			—8.05		
	II	19.34		8.23	
			0.18		—14.36
	III	19.52		—6.13	
			—5.95		
	IV	13.57			

in which d represents the third difference. These formulas may be stated as follows:

$$d = (Y_4 + 3Y_2) - (X_1 + 3X_3)$$

and

$$d = (X_4 + 3X_2) - (Y_1 + 3Y_3)$$

The differences in group A are all negative (table 4) which indicates that cows of that group produced more butterfat while being watered indoors than while being watered outdoors. In group B the treatments were presented in the reverse order, so the differences will be positive if the cows produced more butterfat while being watered from bowls in their stalls. Four of the five differences are in fact positive which increases the evidence that cows will produce a greater amount of butterfat in the winter if they are watered from bowls indoors than they will if watered outdoors. This source of variability may be tested for significance by applying to the third differences the extension of "Student's" t-test given in the preceding section. From the differences in column six of table 4 are found:

Symbol	Group A	Group B
Sd	— 69.91	80.02
$\bar{d}$	— 13.98	16.00
Sd^2	1210.89	2133.12
$(Sd)^2/n$	977.48	1280.64
$S(d - \bar{d})^2$	233.41	852.48

$$s = \sqrt{\frac{233.41 + 852.48}{4 + 4}} = 11.65$$

$$t = \frac{16.00 - (-13.98)}{11.65}\sqrt{\frac{(5)\ (5)}{5 + 5}}$$

$$= 4.07 \quad (D.F. = 8)$$

Since this value of t is highly significant, the conclusion may be drawn that the amount of butterfat produced differs with the method of watering as used in this experiment. The mean increase in butterfat was 5 pounds per cow for a 28-day period.

EXPERIMENTS INVOLVING MORE THAN FOUR TEST PERIODS

More than four experimental periods may be used at times but generally will not be practical for the total time necessary for completing the experiment would be too long or the several periods would be too short. When conditions permit the use of more than four test periods the methods outlined above can be expanded easily.

In general, in switchback or reversal tests involving two treatments, k treatment periods (k equal to or greater than 2) and two groups of test individuals (A and B), the significance of the difference between the (k — 1)st differences of groups A and B may be tested by Fisher's extension of "Student's" treatment of the error of a mean.

For convenient reference, the formulas for the (k — 1)st differences for two, three, four and five test periods are given below.

Two test periods: first difference, $-a + b$
Three test periods: second difference, $a - 2b + c$
Four test periods: third difference, $-a + 3b - 3c + d$
Five test periods: fourth difference, $a - 4b + 6c - 4d + e$

The letters a, b, c, etc. represent the observed values in the first, second, third periods, etc.

PART II.

ANALYSIS OF VARIANCE APPLIED TO REVERSAL OR SWITCHBACK TRIALS INVOLVING TWO OR MORE TEST PERIODS

EXPERIMENTS INVOLVING TWO TEST PERIODS

In the rabbit insulin tests (data for which are given in table 1), used as the first illustration in Part I, there are three sources of variability which may be tested for significance: (a) Type of insulin, (b) day of treatment or period, (c) differences between rabbits. In Part I only the significance of the difference between the percent blood sugar reduction produced by the two types of insulin was tested. All three sources of variability will be tested by the analysis of variance, and the fact that the test of significance between types of insulin is identical whether "Student's" t-test or the analysis of variance is used will be verified.

The subdivisions of the total number of degrees of freedom which specify the structure of the insulin test experiment are given in table 6.

TABLE 6. RABBIT INSULIN TESTS.
(Subdivisions of degrees of freedom specifying structure of experiment.)

Subdivision	Symbol	Degrees of freedom	Sub-total
Period	P	1	1
Individuals:			
Rabbits in Group A	R_A	14	
Rabbits in Group B	R_B	14	
Groups	G	1	29
Interactions:			
Between period and rabbits in Group A	$P \times R_A$	14	
Between period and rabbits in Group B	$P \times R_B$	14	
Between period and group	$P \times G$	1	29
Total		59	59

The arrangement of data and the sums and differences shown in table 1 greatly facilitate the calculation of the total sum of squares and its various subdivisions that are appropriate to the subdivisions of the total number of degrees of freedom given in table 6. These various sums of squares can be calculated conveniently by following the arithmetic rules given below.

Total sum of squares

$$= \text{sum of squares of individual observations} - \frac{(\text{sum of observations})^2}{\text{total number}}$$

$$= 70064 - \frac{(1984)^2}{60} = 4459.7$$

Portion of total sum of squares attributable to period

$$= \frac{(\text{first period total})^2 + (\text{2nd period total})^2}{\text{number in period}} - \frac{(\text{sum of observations})^2}{\text{total number}}$$

$$= \frac{(1109)^2 + (875)^2}{30} - \frac{(1984)^2}{60} = 912.6$$

The remaining portions of the total sum of squares are calculated from group and individual totals instead of from totals for the entire table.

Portion of total sum of squares due to differences between rabbits in a group

$$= \frac{(\text{1st rabbit total})^2 + \ldots + (\text{15th rabbit total})^2}{\text{number of periods}} - \frac{(\text{group total})^2}{\text{number in group}}$$

Thus:
(1) Between rabbits in Group A:

$$\frac{(56)^2 + (60)^2 + (98)^2 + \ldots + (57)^2 + (62)^2}{2} - \frac{(956)^2}{30} = 1748.5$$

where the first rabbit total is 29 + 27 = 56, etc.
(2) Between rabbits in Group B:

$$\frac{(77)^2 + (75)^2 + (59)^2 + \ldots + (64)^2 + (66)^2}{2} - \frac{(1028)^2}{30} = 488.9$$

Portion attributable to difference between groups

$$= \frac{(\text{1st group total})^2 + (\text{2nd group total})^2}{\text{number in group}} - \frac{(\text{grand total})^2}{\text{total number}}$$

$$= \frac{(530 + 426)^2 + (579 + 449)^2}{30} - \frac{(1984)^2}{60} = 86.4$$

The portions of the total sum of squares due to various interactions are calculated from the differences in a similar manner.

Portion attributable to interaction between periods and rabbits in a group

$$= \frac{(\text{1st rabbit difference})^2 + \ldots + (\text{15th rabbit difference})^2}{\text{number periods}} - \frac{(\text{sum differences})^2}{\text{number in group}}$$

Thus:
(1) Interaction between period and rabbits in Group A:

$$\frac{(2)^2 + (18)^2 + (-8)^2 + \ldots + (7)^2 + (2)^2}{2} - \frac{(104)^2}{30} = 430.5$$

(2) Interaction between period and rabbits in Group B:

$$\frac{(7)^2 + (19)^2 + (1)^2 + \ldots + (18)^2 + (4)^2}{2} - \frac{(130)^2}{30} = 781.7$$

Portion due to interaction between period and group:

$$\frac{(\text{1st group total of differences})^2 + (\text{2nd group total of differences})^2}{\text{number in group}}$$

$$- \frac{(\text{grand total of differences})^2}{\text{total observations}} = \frac{(104)^2 + (130)^2}{30} - \frac{(234)^2}{60} = 11.3$$

The mean square for each subdivision of the degrees of freedom is the ratio of the sum of squares to the corresponding number of degrees of freedom. The full analysis of variance follows in table 7.

TABLE 7. RABBIT INSULIN TESTS.
(Analysis of variance.)

	Symbol	D. F.	Sums of squares	Mean square
Period	P	1	912.6	912.6
Individuals:				
Rabbits in Group A	R_A	14	1748.4	124.9
Rabbits in Group B	R_B	14	488.9	34.9
Groups	G	1	86.4	86.4
Total individual	I	29	2323.7	80.1
Interactions:				
Period and rabbits in Group A	$P \times R_A$	14	430.4	
Period and rabbits in Group B	$P \times R_B$	14	781.7	
Period and group	$P \times G$	1	11.3	
Total interaction	$P \times I$	29	1223.4	42.2
Total		59	4459.7	

With the information given in table 7, it is now possible to determine the significance of the effects due to kind of insulin, to period and to differences between rabbits.

Owing to the reversal feature of the design, the effect due to kind of insulin is the interaction between period and group. That this is true is apparent when the treatment totals are arranged in a 2×2 table according to period and group, thus,

Group	Period I	Period II	Totals
A	X_1	Y_2	$X_1 + Y_2$
B	Y_1	X_2	$Y_1 + X_2$
Totals	$X_1 + Y_1$	$Y_2 + X_2$	$X_1 + Y_1 + X_2 + Y_2$

The sum of squares due to interaction is found from the diagonal sums and the grand total by means of the expression

$$\frac{(X_1 + X_2)^2 + (Y_1 + Y_2)^2}{2(n_A + n_B)} - \frac{(X_1 + Y_1 + X_2 + Y_2)^2}{2(n_A + n_B)}$$

or by means of the algebraically equivalent but much simpler expression

$$\frac{[(X_1 + X_2) - (Y_1 + Y_2)]^2}{2(n_A + n_B)}$$

In table 1, $X_1 = 530$, $Y_1 = 579$, $Y_2 = 426$, $X_2 = 449$ and $n_A = n_B = 15$. Substituting these values in the expression immediately above yields 11.3 for the sum of squares for interaction between period and group, which agrees with the value obtained previously.

The estimate of error for testing the significance of the variability due to type or kind of insulin is obtained by pooling the interactions between period and rabbit in the two groups as shown in table 8.

TABLE 8. RABBIT INSULIN TESTS.
(Analysis of effect of kind of insulin.)

	D. F.	Sum of squares	Mean square	.5 $\log_e$
$P \times R_A$	14	430.4		
$P \times R_B$	14	781.7		
	—	—		
Sum (error)	28	1212.1	43.3	1.88
$P \times G$	1	11.3	11.3	1.21

$$Z = 1.21 - 1.88 = -0.67$$

In the case of two classes, the natural logarithm of t is equal to z, thus

$$\log_e t = -0.67$$
$$t = 0.51$$

which checks with the value of t found for this same problem in Part I.

If F (Snedecor, 1937) is defined as the ratio of the variance between classes to that within classes, the functional relation between z and F is

$$F = e^{2z}$$

or

$$\log F = 2z$$

so that, for the case of two classes, $F = t^2$. From the table above, $F = 11.3/43.3 = .26 = (.51)^2$, the square of the value of t found in Part I.

Since the observed difference between the effects of the two insulins is not significant, the significance of the effect of period may be tested. This is done by comparing the mean square corresponding to the 1 degree of freedom for period with that corresponding to the 29 degrees of freedom for interaction between period and individuals. The variances for this comparison are given in table 7, from which

$$F = 912.6/42.2 = 21.6$$

The 1 percent value of F for $n_1 = 1$ and $n_2 = 29$ is 7.60, the observed value of 21.6 being highly significant. The mean of the blood sugar reduction percentages for the 30 rabbits at the first injection is 37.0 and that at the second injection is 29.2. This decrease in percentage reduction of blood sugar induced by later injection of the same quantity of insulin is in agreement with clinical experience with human patients receiving insulin, that is, a tolerance of or resistance to the effect of insulin is built up.

The variability due to individual differences may be tested by comparing the mean square corresponding to the 29 degrees of freedom for individuals with that corresponding to the 29 degrees of freedom for interaction between periods and individuals. From table 7,

$$F = 80.1/42.2 = 1.90$$

By interpolating in the table of F, the 5 percent value is found to be 1.86, this observed value of 1.90 indicating lack of homogeneity. Thus, if this method of measuring the relative efficacy of two insulins is to be used, it would seem that the experimental technique should be improved. Points in technique in design that might be considered are: The test animals, their general suitability for the purpose and their genetic uniformity; operative technique; critical dosages considering age, weight, sex and parentage; adequacy of the sample.

EXPERIMENTS INVOLVING THREE TEST PERIODS

In experiments involving three test periods the total number of degrees of freedom is one less than three times the total number of animals or individuals tested. The structure of the experiment can be specified by properly subdividing the total number of degrees of freedom. In the gluten feed experiment used as an illustration in Part I, the total number of degrees of freedom is (3) (10) — 1 = 29 since 10 cows were used. The subdivisions of these 29 degrees of freedom which specify the structure of this experiment are given in table 9.

TABLE 9. GLUTEN FEED IN A TIMOTHY HAY RATION.
(Subdivision of degrees of freedom specifying structure of experiment.)

Period:			
Linear term	P_1	1	
Quadratic term	P_2	1	2
Individuals:			
Cows in group A	C_A	4	
Cows in group B	C_B	4	
Groups	G	1	9
Interactions:			
Between linear term and cows in group A	$P_1 \times C_A$	4	
Between linear term and cows of group B	$P_1 \times C_B$	4	
Between linear term and group	$P_1 \times G$	1	9
Interactions:			
Between quadratic term and cows in group A	$P_2 \times C_A$	4	
Between quadratic term and cows in group B	$P_2 \times C_B$	4	
Between quadratic term and group	$P_2 \times G$	1	9
Total		29	29

From the data in table 2 the total sum of squares and the sums of squares corresponding to the various subdivisions of the total degrees of freedom in table 9 may be calculated. Those calculations which are identical with the ones given in the preceding example follow without description, but those that differ are given rather fully. The differences in calculation arise, of course, from the fact that three test periods are involved in the present experiment instead of two as in the previous experiment.

Total sum of squares:

$$16487322.03 - \frac{(21539.9)^2}{30} = 1021746$$

Since there are three experimental periods there are two independent comparisons or degrees of freedom between period totals. It seems logical in this case to compare the first period total with the total of the last period for 1 degree of freedom and to compare the sum of the totals for the first and last periods with twice the total for the second period for the second degree of freedom. The first of these 2 degrees of freedom may be designated as the linear term and the second as the quadratic term.

The sum of squares for the linear term (P_1) is a fraction of the square of the difference between the totals for the first and third periods. The denominator of the fraction is the product of the number of observations entering into each period total and the sum of the squares of the coefficients of the terms in the numerator.

$$P_1 = \frac{(7597.5 - 6761.1)^2}{10\,(1^2 + 1^2)}$$
$$= 34978$$

The sum of squares for the quadratic term (P_2) is a fraction of the square of the difference between the totals for the first and third periods and twice the total for the second period. The denominator of the fraction is the product of the number of observations entering into each period total and the sum of the squares of the coefficients of the terms in the numerator.

$$P_2 = \frac{[7597.5 + 6761.1 - (2)\,(7181.3)]^2}{10(1^2 + 1^2 + 2^2)} = 0$$

Individuals:

Sum of squares for cows of group A (C_A):

$$\frac{(1209.6)^2 + (2322.3)^2 + (2292.1)^2 + (3009.8)^2 + (1765.7)^2}{3}$$
$$- \frac{(10599.5)^2}{15} = 605548$$

Sum of squares for cows of group B (C_B):

$$\frac{(1878.4)^2 + (1659.0)^2 + (2203.8)^2 + (3000.2)^2 + (2199.0)^2}{3}$$
$$- \frac{(10940.4)^2}{15} = 345244$$

Sum of squares for group (G):

$$\frac{(10599.5)^2 + (10940.4)^2}{15} - \frac{(21539.9)^2}{30} = 3874$$

Interactions:

The portions of the total sum of squares attributable to the various interactions are calculated from differences computed from table 2 as follows:

Between linear term and cows of group A ($P_1 \times C_A$):

$$\frac{[(70.1)^2+(-35.7)^2+(178.1)^2+(-30.2)^2+(160.4)^2]-(342.7)^2/5}{(1^2+1^2)}$$

$= 20530$

the first difference being $433.0 - 362.9 = 70.1$.

Between linear term and cows of group B ($P_1 \times C_B$):

$$\frac{[(74.5)^2+(126.6)^2+(83.0)^2+(162.2)^2+(47.4)^2]-(493.7)^2/5}{(1^2+1^2)}$$

$= 4137$

Between linear term and group ($P_1 \times G$):

$$\frac{[(342.7)^2+(493.7)^2]-(836.4)^2/2}{5(1^2+1^2)} = 1140$$

where $342.7 = 3668.0 - 3325.3$.

Between quadratic term and cows of group A ($P_2 \times C_A$):

$$\frac{[(-31.5)^2+(-69.9)^2+(30.7)^2+(-65.8)^2+(-82.6)^2]-(-219.1)^2/5}{(1^2+2^2+1^2)}$$

$= 1395$

Between quadratic term and cows of group B ($P_2 \times C_B$):

$$\frac{[(47.5)^2+(-7.2)^2+(4.8)^2+(46.2)^2+(123.8)^2]-(215.1)^2/5}{(1^2+2^2+1^2)}$$

$= 1756$

Between quadratic term and group ($P_2 \times G$):

$$\frac{[(-219.1)^2+(215.1)^2]-(-4.0)^2/2}{5(1^2+2^2+1^2)} = 3142$$

The analysis of variance summarizing these calculations is given in table 10.

TABLE 10. GLUTEN FEED IN A TIMOTHY HAY RATION.
(Analysis of variance.)

	D. F.	Sums of squares	Mean square
Periods:			
P_1	1	34978	34978
P_2	1	0	0
Individuals:			
C_A	4	605548	151387
C_B	4	345244	86311
G	1	3874	3874
Interactions:			
$P_1 \times C_A$	4	20530	5132
$P_1 \times C_B$	4	4137	1034
$P_1 \times G$	1	1140	1140
Interactions:			
$P_2 \times C_A$	4	1395	349
$P_2 \times C_B$	4	1756	439
$P_2 \times G$	1	3142	3142
	29	1021746*	

*The slight discrepancy is due to rounding.

Owing to the double reversal or switch-back feature of the design, the effect due to grain mixture is identical with the interaction between the quadratic term and group ($P_2 \times G$). That this is true is easily demonstrated by the following 2×2 table.

	Period	
	Quadratic term	
Group	I + III	(2) II
A	$X_1 + X_3$	$2Y_2$
B	$Y_1 + Y_3$	$2X_2$

The interaction between the quadratic term and group is the diagonal comparison in the above table, that is, it is a function of the expression $(X_1 + 2X_2 + X_3) - (Y_1 + 2Y_2 + Y_3)$. This expression is also the difference between results obtained from rations X and Y properly weighted to make efficient use of all available information.

In this experiment, owing to the design, there is but one relevant question—the significance of the variability due to grain mixture which, as has just been shown, is the same as the interaction between quadratic term and group. The appropriate estimate of error for testing the significance of this interaction is made by pooling the interaction between quadratic term and individuals in the two groups. The analysis is given in table 11.

TABLE 11. GLUTEN FEED IN A TIMOTHY HAY RATION.
(Analysis of effect of grain mixture)

	D. F.	Sums of squares	Mean square
$P_2 \times C_A$	4	1395	
$P_2 \times C_B$	4	1756	
Sum	8	3151	394
$P_2 \times G$	1	3142	3142

$$F = 3142/394 = 7.97$$

Since for two classes, $F = t^2$, a value of F can also be determined from the value of t found in Part I. There it was found that

$$t = 2.82.$$

Hence

$$F = (2.82)^2 = 7.95$$

the small discrepancy being due to rounding. Thus, it is seen, results of the analysis of variance and of the extension of "Student's" t-test are identical. The discussion of this result was given in Part I.

EXPERIMENTS INVOLVING FOUR TEST PERIODS

In an experiment involving four test periods, the total number of degrees of freedom is one less than four times the total number of individuals involved. In the experiment on the use of water bowls in the dairy barn, Cannon, Hansen and O'Neil (2), used as an illustration in Part I, there are 39 degrees of freedom. These 39 degrees of freedom may be subdivided according to the design of the experiment, as given in table 12.

TABLE 12. USE OF WATER BOWLS IN THE DAIRY BARN.
(Subdivisions of degrees of freedom specifying structure of experiment.)

Periods:			
Linear term	P_1	1	
Quadratic term	P_2	1	
Cubic term	P_3	1	3
Individuals:			
Cows in group A	C_A	4	
Cows in group B	C_B	4	
Groups	G	1	9
Interactions:			
Between linear term and cows of group A	$P_1 \times C_A$	4	
Between linear term and cows of group B	$P_1 \times C_B$	4	
Between linear term and group	$P_1 \times G$	1	9
Interactions:			
Between quadratic term and cows of group A	$P_2 \times C_A$	4	
Between quadratic term and cows of group B	$P_2 \times C_B$	4	
Between quadratic term and group	$P_2 \times G$	1	9
Interactions:			
Between cubic term and cows of group A	$P_3 \times C_A$	4	
Between cubic term and cows of group B	$P_3 \times C_B$	4	
Between cubic term and group	$P_3 \times G$	1	9
Total		39	39

The only pertinent question in this experiment is the significance of the variability due to the method of watering. This is identical with the interaction between the cubic term and group as is shown by the following 2×2 table.

	Period cubic term	
Group	I + (3) III	(3) II + IV
A	$X_1 + 3X_3$	$3Y_2 + Y_4$
B	$Y_1 + 3Y_3$	$3X_2 + X_4$

The interaction between cubic term and group is determined from the diagonals of the above table, that is, it is a function of the expression $(X_1 + 3X_2 + 3X_3 + X_4) - (Y_1 + 3Y_2 + 3Y_3 + Y_4)$. This expression is also the yield of butterfat of the 10 cows while being watered indoors from bowls minus the yield of the same cows being watered outdoors from a tank which is, of course, the difference the experiment was designed to test. The error variance appropriate for testing the interaction between cubic term and group is obtained by pooling the interaction between cubic term and cows of group A with that between cubic term and cows of group B. Thus, only the three portions of the total sum of squares which correspond to these three subdivisions of the total number of degrees of freedom need be calculated. Using the third differences in column 6 of table 4, the calculations are:

Interaction between cubic term and cows of group A

$(P_3 \times C_A)$:

$$\frac{(-14.91)^2 + (-14.36)^2 + (-21.73)^2 + (-1.35)^2 + (-17.56)^2 - (-69.91)^2/5}{1^2 + 3^2 + 3^2 + 1^2}$$

$$= 11.67$$

Interaction between cubic term and cows of Group B $(P_3 \times C_B)$:

$$\frac{(34.62)^2 + (-5.16)^2 + (21.70)^2 + (11.22)^2 + (17.64)^2 - (80.02)^2/5}{1^2 + 3^2 + 3^2 + 1^2}$$

$$= 42.62$$

Interaction between cubic term and group $(P_3 \times G)$:

$$\frac{(-69.91)^2 + (80.02)^2 - (10.11)^2/2}{5(1^2 + 3^2 + 3^2 + 1^2)} = 112.40$$

A check on the accuracy of the above three calculations may be made by calculating the interaction between cubic term and all cows $(P_3 \times C)$, which should be the sum of the previous three results. Thus

$$\frac{(-14.91)^2 + (-14.36)^2 + \ldots + (11.22)^2 + (17.64)^2 - (10.11)^2/10}{1^2 + 3^2 + 3^2 + 1^2}$$

$$= 166.69$$

Check:

$$11.67 + 42.62 + 112.40 = 166.69$$

The relevant portion of the analysis of variance is given in table 13.

TABLE 13. EFFECT OF METHOD OF WATERING ON PRODUCTION OF BUTTERFAT.
(Analysis of variance.)

	D. F.	Sums of squares	Mean squares
$P_3 \times C_A$	4	11.67	
$P_3 \times C_B$	4	42.62	
$(P_3 \times C_A) + (P_3 \times C_B)$	8	54.29	6.79
$P_3 \times G$	1	112.40	112.40
Total	9	166.69	

$$F = 112.40/6.79 = 16.56$$

In Part I, using these same data, t was found to be 4.07. F calculated from this value of t ($F = t^2$ for two classes) is found to be 16.56. The method of analysis of variance and the extension of "Student's" t-test proposed in this paper have produced identical results for an experiment involving four test periods, just as they did for experiments involving two and three test periods.

In many experiments, but one measure concerning the test animals is available. Under such conditions the above analysis is all that is possible, but Cannon, Hansen and O'Neil (2) have recorded information concerning the pounds of milk produced and the gallons of water consumed in addition to that on the pounds of butterfat produced. These data, together with their third differences, are given in tables 14 and 15.

TABLE 14. USE OF WATER BOWLS IN THE DAIRY BARN.

(Production of milk in pounds.)

Cow number	Period I	Period II	Period III	Period IV	Third differences
Group A					
	X	Y	X	Y	
906	1179.2	1027.8	802.9	384.1	—120.4
750	850.4	698.1	610.3	431.5	—155.5
787	1048.4	946.8	934.9	841.2	—171.5
796	619.7	566.4	549.2	505.1	— 63.0
933	824.6	687.6	690.0	615.8	—216.0
Sums	4522.3	3926.7	3587.3	2777.7	—726.4
Group B					
	Y	X	Y	X	
749	960.1	802.6	771.6	752.6	—114.5
817	577.5	558.1	511.5	497.2	59.5
675	1113.2	1094.0	930.4	910.9	288.5
833	963.5	807.5	752.7	680.9	—118.2
763	743.4	640.8	464.3	437.9	224.0
Sums	4357.7	3903.0	3430.5	3279.5	339.3
Both groups					
Sums	8880.0	7829.7	7017.8	6057.2	—387.1

TABLE 15. USE OF WATER BOWLS IN THE DAIRY BARN.

(Gallons of water consumed.)

Cow number	Period I	Period II	Period III	Period IV	Third differences
Group A					
	X	Y	X	Y	
906	477.6	356.2	369.3	316.7	—200.2
750	324.7	254.8	288.4	267.3	—158.2
787	380.7	294.4	366.5	308.9	—288.1
796	260.9	184.5	222.6	211.4	—163.8
933	293.4	253.1	272.7	250.8	—101.4
Sums	1737.3	1343.0	1519.5	1355.1	—911.7
Group B					
	Y	X	Y	X	
749	291.1	287.4	264.8	336.6	113.3
817	200.8	218.5	196.7	230.6	95.2
675	364.7	427.9	323.1	391.8	341.5
833	314.6	343.9	291.4	355.4	198.3
763	240.0	286.8	193.3	277.9	318.4
Sums	1411.2	1564.5	1269.3	1592.3	1066.7
Both groups					
Sums	3148.5	2907.5	2788.8	2947.4	155.0

In setting up this experiment an attempt was made to balance the two groups of cows as nearly as possible; however, the variation in the individual yields of milk given in table 14 is considerable. If warranted, this source of variability may be eliminated from the estimates of error by the methods of the analysis of covariance, section 49.1 in Fisher (4). For the analysis of covariance the sums of squares of pounds of milk and the sums of cross products of pounds of milk by pounds of butterfat corresponding to the three interactions in table 13 must be calculated.

Interaction between cubic terms and cows of group A ($P_3 \times C_A$):

(a) Sum of squares (pounds of milk):

$$\frac{(-120.4)^2+(-155.5)^2+(-171.5)^2+(-63.0)^2+(-216.0)^2-(-726.4)^2/5}{1^2+3^2+3^2+1^2}$$

$$= 659.11$$

(b) Sum of cross products (pounds of milk by pounds of butterfat):

$$\frac{(-120.4)(-14.91)+\ldots+(-216.0)(-17.56)-(-726.4)(-69.91)/5}{1^2+3^2+3^2+1^2}$$

$$= 73.82$$

Interaction between cubic term and cows of group B ($P_3 \times C_B$):

(a) Sum of squares (pounds of milk):

$$\frac{(-114.5)^2+(59.5)^2+(288.5)^2+(-118.2)^2+(224.0)^2-(339.3)^2/5}{1^2+3^2+3^2+1^2}$$

$$= 7050.25$$

(b) Sum of cross products (pounds of milk by pounds of butterfat):

$$\frac{(-114.5)(34.62)+\ldots+(224.0)(17.64)-(339.3)(80.02)/5}{1^2+3^2+3^2+1^2}$$

$$= -40.78$$

Interaction between cubic term and group ($P_3 \times G$):

(a) Sum of squares (pounds of milk):

$$\frac{(-726.4)^2+(339.3)^2-(-387.1)^2/2}{5(1^2+3^2+3^2+1^2)} = 5678.58$$

(b) Sum of cross products (pounds of milk by pounds of butterfat):

$$\frac{(-726.4)(-69.91)+(339.3)(80.02)-(-387.1)(10.11)/2}{5(1^2+3^2+3^2+1^2)}$$

$$= 798.90$$

Interaction between cubic term and all cows ($P_3 \times C$):

(a) Sum of squares (pounds of milk):

$$\frac{(-120.4)^2+(-155.5)^2+\ldots+(-118.2)^2+(224.0)^2-(-387.1)^2}{1^2+3^2+3^2+1^2}$$

$$= 13387.95$$

Check: 659.11 + 7050.25 + 5678.58 = 13387.94.

(b) Sum of cross products (pounds of milk by pounds of butterfat):

$$\frac{(-14.91)(-120.4)+\ldots+(17.64)(224.0)-(-387.1)(10.11)/10}{1^2+3^2+3^2+1^2}$$

$= 831.94$

Check: 73.82 — 40.78 + 798.90 = 831.94.

The information now available for analyzing the covariance is summarized in table 16.

TABLE 16. EFFECT OF METHOD OF WATERING ON PRODUCTION OF BUTTERFAT.

(Sums of squares and cross-products.)

	D. F.	Milk Sx^2	Milk by butterfat Sxy	Butterfat Sy^2
$(P_3 \times C_A)$	4	659.11	73.82	11.67
$(P_3 \times C_B)$	4	7050.25	—40.78	42.62
$(P_3 \times C_A) + (P_3 \times C_B)$	8	7709.37	33.04	54.29
$(P_3 \times G)$	1	5678.58	798.90	112.40
Total	9	13387.95	831.94	166.69

The sum of squares for butterfat Sy^2 corresponding to the 8 degrees of freedom and that corresponding to the 9 degrees of freedom may be adjusted by subtracting an allowance for the regression of yield of butterfat on yield of milk. The adjusted value is obtained in each case by deducting from the Sy^2 in a line, the quantity $(Sxy)^2/(Sx^2)$ derived from the same line. This is the same as saying that the adjusted value is the product

$(Sy^2)\ (1 - r^2)$, because

$$(Sy^2) - \frac{(Sxy)^2}{(Sx^2)} = \left[(Sy^2) - \frac{(Sxy)^2}{(Sx^2)} \right] \frac{(Sy^2)}{(Sy^2)}$$

$$= (Sy^2) \left[1 - \frac{(Sxy)^2}{(Sx^2)\ (Sy^2)} \right]$$

$$= (Sy^2)\ (1 - r^2)$$

The latter form entails more calculation and should be used only in case the values of r have been computed for some other purpose.

The sum of squares corresponding to the remaining degree of freedom is found by subtracting the adjusted sum of squares for the 8 degrees of freedom from that corresponding to the 9 degrees of freedom. In making the test of significance, this value is to be compared to the corrected mean square for the 8 degrees of freedom. This procedure is given in table 17.

The value of F, 7.86 is significant though not highly so. Without this adjustment for regression the difference in yield of butterfat was found to be highly significant, table 13. Though the cows in the two groups differed considerably with regard to the amount of milk produced, there still remains a significant differ-

TABLE 17. EFFECT OF METHOD OF WATERING ON PRODUCTION OF BUTTERFAT.
(Test of significance with adjusted variance.)

	D. F.	Adjusted sum of squares	Adjusted mean square
$(P_3 \times C_A) + P_3 \times C_B)$	7	54.15	7.74
$(P_3 \times G)$	1	60.84	60.84
Total	8	114.99	

ence in yield of butterfat after this element of heterogeneity has been eliminated. Thus it appears that even if the groups had been perfectly balanced as to production of milk, the two methods of watering would have produced a significant difference in yield of butterfat.

The method of covariance can be extended to more than two variates and their covariances so as to make adjustment simultaneously for two or more measurable but uncontrolled factors. The information on gallons of water consumed given in table 15 may thus be combined with that for yield of milk and yield of butterfat in what might be designated as a multiple covariance analysis. The multiple correlation methods given by Wallace and Snedecor (7) and by Ezekiel (3), with a few minor changes, are used for calculating the sums of squares and sums of products of the third differences in tables 4, 14 and 15 and for calculating the necessary multiple correlation coefficients. These calculations are given in tables 18 and 19.

TABLE 18. INTERACTION BETWEEN CUBIC TERM AND COWS OF GROUP A ($P_3 \times C_A$).
(Calculation of sums of squares and of cross-products.)

		Water consumed	Milk produced	Butterfat produced
Sums		—911.7	—726.4	—69.91
Means		—182.34	—145.28	—13.98
Water	1	185221.29	130335.13	13518.86
	2	166239.38	132451.78	12747.39
	3	18981.91	—2116.65	771.47
	4	949.10	—105.83	38.574
Milk	1		118713.66	11632.85
	2		105531.39	10156.52
	3		13182.27	1476.32
	4		659.11	73.816
Butterfat	1			1210.89
	2			977.48
	3			233.41
	4			11.670

Divisor = 20

The two rows at the top and the first three rows in each block in tables 18, 19, 20 and 21 agree exactly with the corresponding

TABLE 19. INTERACTION BETWEEN CUBIC TERM AND COWS OF GROUP B ($P_3 \times C_B$).
(Calculation of sums of squares and of cross-products.)

		Water consumed	Milk produced	Butterfat produced
Sums		1066.7	339.3	80.02
Means		213.34	67.86	16.00
Water	1	279223.63	139096.84	18683.27
	2	227569.78	72386.26	17071.47
	3	51653.85	66710.58	1611.80
	4	2582.69	3335.53	80.590
Milk	1		164029.99	4614.60
	2		23024.90	5430.16
	3		141005.09	—815.56
	4		7050.25	—40.778
Butterfat	1			2133.12
	2			1280.64
	3			852.48
	4			42.624

Divisor = 20

TABLE 20. INTERACTION BETWEEN CUBIC TERM AND GROUP ($P_3 \times G$).
(Calculation of sums of squares and of cross-products.)

		Water consumed	Milk produced	Butterfat produced
Sums		155.0	—387.1	10.11
Means		77.5	—193.55	5.06
Water	1	1969045.78	1024190.19	149094.28
	2	12012.50	—30000.25	783.52
	3	1957033.28	1054190.44	148310.76
	4	19570.33	10541.90	1483.11
Milk	1		642781.45	77933.41
	2		74923.20	—1956.79
	3		567858.25	79890.20
	4		5678.58	798.90
Butterfat	1			11290.61
	2			51.11
	3			11239.50
	4			112.40

Divisor = 100

parts of table 88 in Ezekiel (3) or table 7 in Wallace and Snedecor (7) and are fully explained there. An entry in line four of any table is obtained by dividing the corresponding entry in line three by the product of the number of quantities entering into an item and the sum of the squares of the coefficients in the function used to calculate the quantities. The quantities in this case are third differences and were calculated from the function $-a + 3b - 3c + d$, in which a, b, c and d represent the measured

TABLE 21. INTERACTION BETWEEN CUBIC TERM AND ALL COWS ($P_3 \times C$).
(Calculation of sums of squares and of cross-products.)

		Water consumed	Milk produced	Butterfat produced
Sums		155.0	—387.1	10.11
Means		15.5	—38.71	1.01
Water	1	464444.92	269431.97	32202.13
	2	2402.50	—6000.05	156.70
	3	462042.42	275432.02	32045.43
	4	23102.12	13771.60	1602.27
Milk	1		282743.65	16247.44
	2		14984.64	—391.36
	3		267759.01	16638.80
	4		13387.95	831.94
Butterfat	1			3344.00
	2			10.22
	3			3333.78
	4			166.69

Divisor = 20

observation for the first, second, third and fourth periods respectively. The coefficients are —1, 3, —3, and 1, and the sum of their squares is 20. This is one factor in the divisor for each of the four tables, the other factor being one in all except table 20 in which it is five because group totals are used, and each group contains five cows. The product of these two factors, called the divisor, is given at the bottom of each table.

The adjusted sum of squares for the error and for the total in a multiple covariance problem is the product in each case of the sum of squares and the quantity $(1 - R^2)$ derived from the same line of the table, R being the multiple correlation coefficient. The procedure of adjusting the sums of squares in a multiple covariance table, then, is the same as that in a covariance table except

TABLE 22. POOLED SUM OF SQUARES FOR ERROR ($P_3 \times C_A$) + ($P_3 \times C_B$).
(Calculation of correction factor.)

		Water	Milk	Butterfat
Water	1	3531.8	3229.7	119.16
	2	—1.0	—.91446	—.03374
Milk	3		7709.4	33.04
	4		—2953.4	—108.97
	5		4756.0	—75.93
	6		—1.0	.01597
Milk			—.01597	—.01597
Water		.04834	.01460	.03374

$$R^2 = \frac{(.04834)\ (119.16) - (.01597)\ (33.04)}{54.29} = .0964$$

$$1 - R^2 = .9036$$

that in the correction factors the simple correlation coefficient is replaced by the multiple correlation coefficient. In any of the standard methods for calculation, the multiple correlation coefficient may be used, those presented by Wallace and Snedecor (7) or by Ezekiel (3) being convenient. In this illustration, the method given by Ezekiel in table 89 is used except for the back solution which follows the arrangement presented by Wallace and Snedecor in table 8. The calculations for the factors for correcting or adjusting the sums of squares of the dependent variable, pounds of butterfat, are given in tables 22 and 23.

TABLE 23. INTERACTION BETWEEN CUBIC TERM AND ALL COWS ($P_3 \times C$).
(Calculation of correction factor.)

		Water	Milk	Butterfat
Water	1	23102	13772.	1602.3
	2	—1	—.59612	—.06936
Milk	3		13388.	831.94
	4		—8209.5	—955.14
	5		5178.5	—123.20
	6		—1.0	.02379
Milk			—.02379	—.02379
Water		.08354	.01418	.06936

$$R^2 = \frac{(.08354)\ (1602.3) - (.02379)\ (831.94)}{166.69} = .6843$$

$$1 - R^2 = .3157$$

The procedures of adjusting the sums of squares for butterfat with the factors just calculated and the test of significance are given in table 24.

TABLE 24. EFFECT OF METHOD OF WATERING ON PRODUCTION OF BUTTERFAT.
(Test of significance with adjusted variance.)

	D. F.	Sums of squares (Sy^2)	Correction factor $1 - R^2$	D.F.	Adjusted sums of squares (Sy^2) ($1-R^2$)	Adjusted variance
Error	8	54.29	.9036	6	49.06	8.18
$P_3 \times G$	1	112.40		1	3.56	3.56
Total	9	166.69	.3157	7	52.62	

It thus appears that the regression of yield of butterfat on yield of milk and amount of water consumed accounts for the observed difference in pounds of butterfat produced by cows while being watered from water bowls in their stalls and while being watered from a tank outside. In other words, the effect of the different methods of watering on the yield of butterfat apparently was a consequence of the effect of these methods on the amount of water consumed and the amount of milk produced.

Methods have been presented for analyzing the results of switchover or reversal trials. The methods have been illustrated by problems involving two, three and four test periods. More than four test periods may be used but will not be practical, generally, for the total time for the experiment would be too great, or the several periods would be too short. If conditions permit the use of more than four test periods, the methods outlined above may be extended easily, and, if the number of individuals is great enough, the number of independent variables may be increased.

SOURCES OF DATA AND METHODS

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